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A new record of the freshwater polychaete *Namanereis hummelincki* (Polychaeta: Nereididae) from epigean waters of Montserrat

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Abstract

The presence of the freshwater polychaete, *Namanereis hummelincki* (Augener), on Montserrat is documented for the first time. Although collected in the sediment of a freshwater stream, this subterranean species most likely lives in groundwater aquifers. A mitochondrial cytochrome c oxidase subunit I (COI) sequence obtained from this material supports assignment to the genus *Namanereis* Chamberlin, and morphological analysis supports identification as *N. hummelincki*. Differences in jaw morphology observed in the Montserrat specimens may indicate long-term separation from other Caribbean island populations.

Key words: Neotropical, Antilles, Caribbean, Invertebrates, COI

Introduction

Polychaetes (Annelida; Polychaeta) are predominantly marine organisms, however over their evolutionary history, a number of taxa have invaded estuarine and freshwater habitats. There are at least 168 species of freshwater polychaete from 12 different families (Glasby & Timm 2008). The family with the greatest number of described freshwater species is the Nereididae, within which there is a mostly-freshwater subfamily, the Namanereidinae, comprehensively revised by Glasby (1999). Atypical freshwater habitats for polychaetes include subterranean waters (Glasby & Timm 2008), where they are often eyeless and lack epidermal pigmentation (Glasby 1999).

Here, we report the presence of a freshwater polychaete from the epigean waters of Montserrat, Lesser Antilles. We provide morphological evidence supporting the identification of this organism as *Namanereis hummelincki* (Augener), new to the known fauna of the island.

Study area

Montserrat is a small volcanic island near the northern end of the Lesser Antilles (Fig. 1). The Nantes River is located on the southwestern side of the island (Fig. 1) and is the first intact drainage north of the pyroclastic flow and subsequent lahars that buried the island's largest freshwater stream, the Belham River, during the volcanic eruptions of 1995–2012 (Barclay *et al.* 2007).

Like most of the freshwater drainages on Montserrat (locally called “ghauts”), the Nantes River has many boulders and large cobble in the stream bed and flows in a ravine with steep walls. The Nantes River is about 3 km long and drops about 550 m in altitude (Montserrat Tourist Board 1983). This river is fed by springs, the largest of which produces 12 L/s located at 255 m above sea level (Hemmings *et al.* 2015). There is a seasonal pattern to rainfall on the island, and the local wet season spans from July to November (Hemmings *et al.* 2015). In January 2015, the Nantes River was flowing, though it was shallow and no more than 1 m wide. Pools (up to 2.5 m wide, 1 m deep) alternated with very shallow riffles and vertical drops up to 2 m. The scoured appearance of the stream bed with minimal leaf litter suggested that there were periods of very high flows in the recent past.

Field methods

During our stream surveys, which were primarily aimed at collecting gastropods, the surfaces of stones, decaying leaves, and detrital deposits were examined in the field by eye. These observations were purely qualitative. Substrate was periodically swept with a small dip net (mesh size 3.5 mm) and sifted by hand. Two freshwater polychaetes were collected in these substrate sweeps. Both were deposited in the National Museum of Natural History of the Smithsonian Institution, Washington, D.C. (USNM). The first polychaete (USNM 1283368) was fixed and preserved in 95% ethanol and the second (USNM 1283369) was fixed and preserved in 10% formalin.

Laboratory methods

Both specimens were examined under a dissecting microscope. The pharynx of the first specimen (USNM 1283368) was dissected by making a 3–4 mm incision dorsomedially behind the prostomium. A jaw piece (Fig. 3) was then removed through this slit, placed on a single depression slide, and mounted using a generic non-drying mounting medium. A parapodium was dissected from a setiger around the middle of the body of the second specimen (USNM 1283369) and was prepared using the same method. Both were observed under 200X power with a light microscope and measured with a stage micrometer.

The specimen preserved in 95% ethanol, USNM 1283368, serves as the first DNA voucher for the species. Genomic DNA was extracted from roughly 1/3 of the animal using a DNeasy Blood & Tissue Kit (QIAGEN) and a 710 bp region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using primers HCO2198 and LCO1490 (Folmer *et al.* 1994). PCR products were purified using a Qiaquick PCR Purification Kit (QIAGEN) and sequenced using the same primers as the PCR (DNA Analysis Facility on Science Hill, Yale University). Complementary forward and reverse sequences were aligned and edited in CodonCode Aligner (CodonCode Corporation, www.codoncode.com), and the resulting sequence was used in a BLAST search of the GenBank nucleotide database (blastn) (Altschul *et al.* 1990). DNA sequences similar to the *Namanereis hummelincki* voucher were downloaded and aligned using ClustalW (Tamura *et al.* 2013). Alignments were edited and poorly aligning flanking regions were removed. Aligned sequences were translated, using an invertebrate mitochondrial genetic code table, into amino acid sequences to check for alignment errors. A neighbor-joining tree (bootstrap, 1000 replications) was constructed including ten COI sequences representing the most similar taxa deposited in GenBank. Pairwise distances (p-distance, complete deletion) were calculated between the nucleotide and amino acid sequences of the Montserrat polychaete and those published sequences that were most similar (Tamura *et al.* 2013).

Results

An individual polychaete was collected in the Nantes River at each of two sampling locations. The first specimen, USNM 1283368, was caught in the Nantes River about 50 m downstream of the Olveston Estate Road bridge, Salem ($16^{\circ}45'08.2''N$ $62^{\circ}13'20.3''W$) on January 1, 2015. The second specimen, USNM 1283369, was caught about 240 m upstream of the first, along Glebe Road, Salem ($16^{\circ}45'11.6''N$ $62^{\circ}13'12.9''W$) on January 17, 2015. These worms were sifted from pockets of soft sediment with bits of organic detritus, a substrate type that was quite rare in the stream bed.

The polychaetes are eyeless and have no epidermal pigmentation. Comparison of the anatomy of the worms to descriptions and illustrations by Glasby (1999) and Williams (2004) supported the identification of these specimens as *Namanereis hummelincki* (Augener).

In particular, the eyeless head (Fig. 2) has two smooth cirriform antennae and three pairs of peristomial tentacular cirri. The jaw (Fig. 3) is terminally bifid with no distinct subterminal or proximally ensheathed teeth. The parapodia lack notopodia and the neuropodia generally have five setae each (two dorsal and three ventral) and never contain more than two aciculae; the dorsal neuropodial setae usually consist of one falciger and one spiniger, and the ventral neuropodial setae consist of two falcigers and one pseudospiniger. No other freshwater polychaete in the Caribbean possesses this combination of characteristics (Glasby 1999).

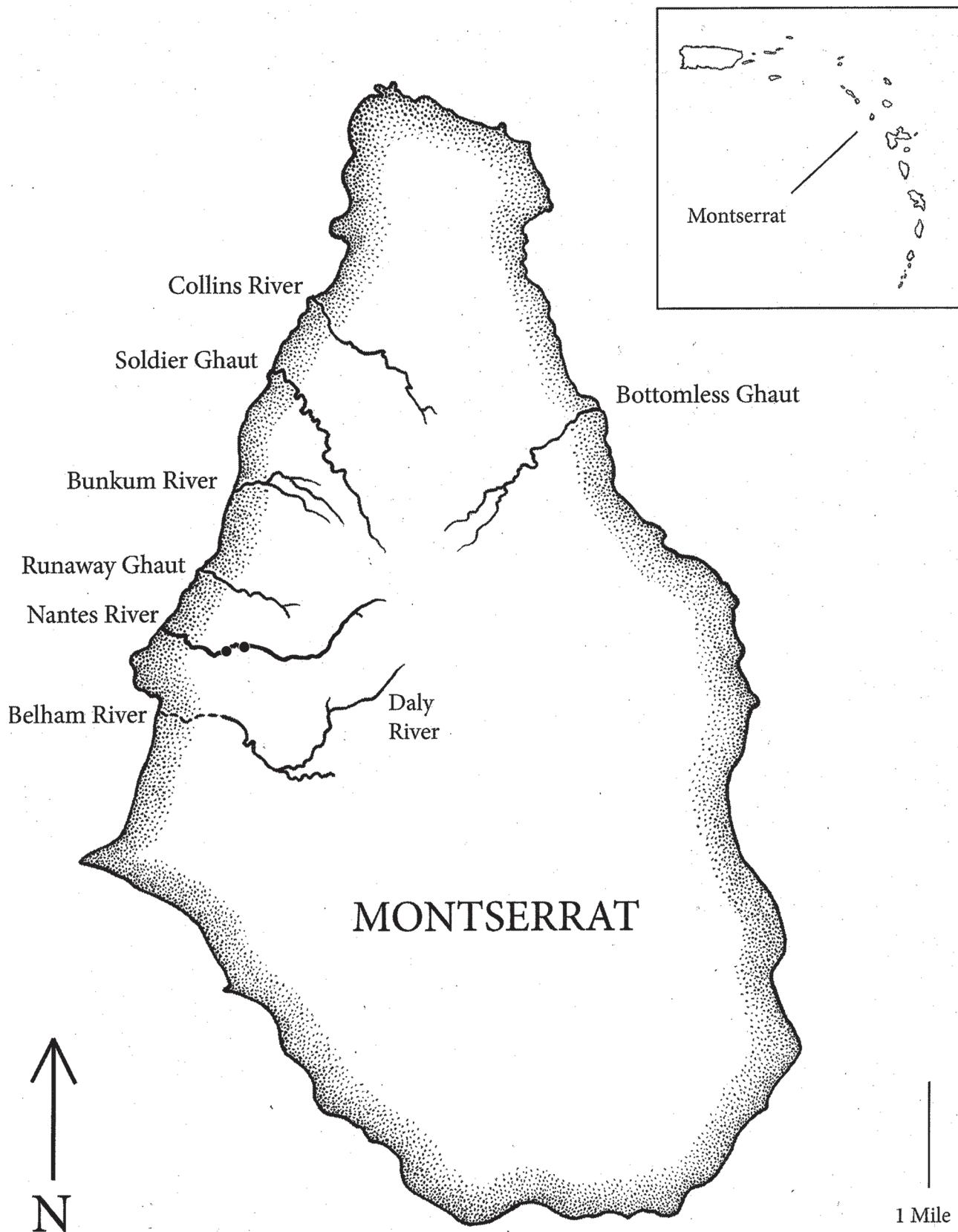


FIGURE 1. Montserrat and its major drainages. The two collection sites mentioned in this paper are marked by small dots along the Nantes River.



FIGURE 2. *Namanereis hummelincki* USNM 1283369; preserved; dorsal view with stage micrometer.

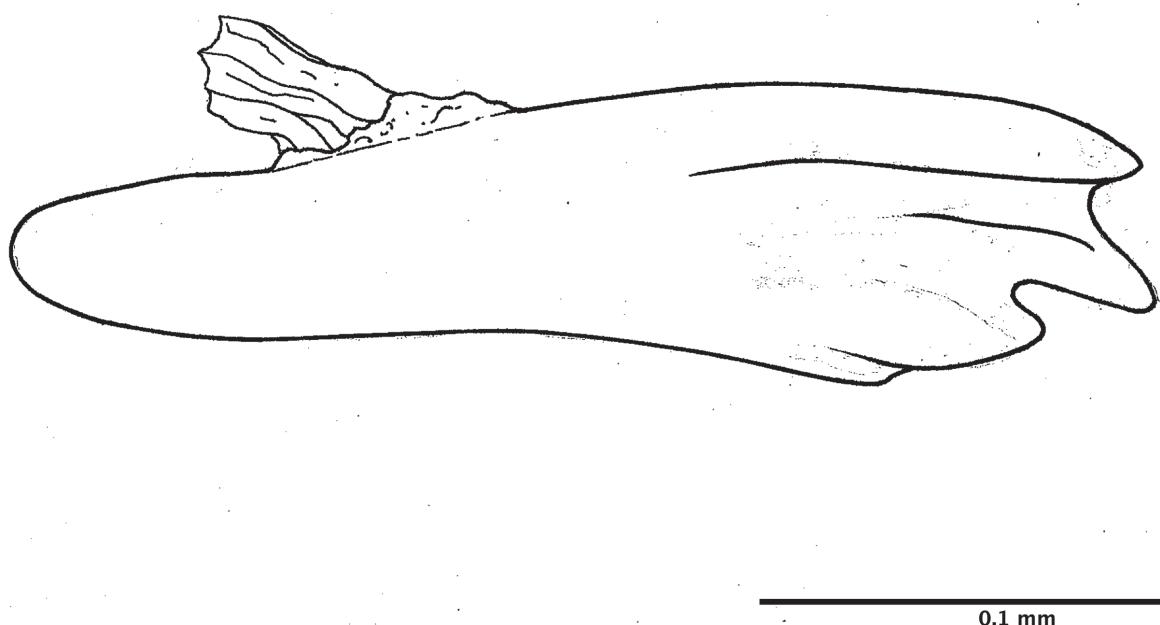


FIGURE 3. Jaw piece dissected from *Namanereis hummelincki* USNM 1283368, ventromedial view. (Note the protrusion slightly proximal to the jaw's terminal bifurcation.)

USNM 1283369 has 90 setigerous segments and is approximately 26 mm long (Fig. 2); USNM 1283368 is too degraded to count setigers and is roughly 23 mm long. These individuals are therefore adult in size, however there are no visible ova, perhaps due to the season when they were collected. The documentation of this species is a new record for the fresh waters of Montserrat.

The GenBank accession number for the COI gene of USNM 1283368; the *Namanereis hummelincki* DNA voucher, is KT235957. The Neighbor-Joining tree (Fig. 4) demonstrates that the Montserrat polychaete COI nucleotide sequence is most similar to sequence data published for *Namanereis* sp. from the Canary Islands, Spain (GenBank Accession numbers JX420279 and JX420280) (Glasby *et al.* 2013), falling within a strongly supported group (bootstrap value = 99%) that also includes three sequences from an undescribed *Namalycastis* species from the Indian coast (JX843801, JX843802, and JX867720), and a *Namalycastis abiuma* Grube sequence, (KF737176). The Montserrat polychaete was more similar to *Namanereis* sp. (pairwise p-distance = 0.181) than to the *Namalycastis* sp. sequences (average pairwise p-distances 0.193), while *Namalycastis* sp. and *Namanereis* sp. sequences were more distant from each other (average p-distance = 0.206). For amino acid sequence comparisons, the COI sequence from the Montserrat polychaete was most similar to *Namanereis* sp. (p-distance = 0.014), and somewhat less similar to *Namalycastis* sp. (p-distance=0.023), while *Namalycastis* sp. and *Namanereis* sp. had a p-distance of 0.037.

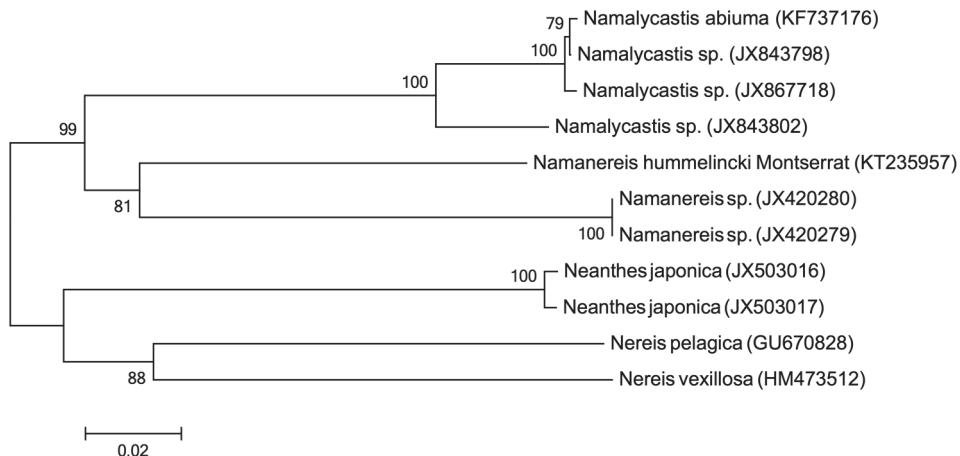


FIGURE 4. Neighborhood-Joining phylogenetic tree of *Namanereis hummelincki* (USNM 1283368) from Montserrat and similar sequences. The evolutionary history was inferred using the Neighborhood-Joining method (Saitou & Nei 1987). The optimal tree with the sum of branch length = 0.66065705 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei & Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 585 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

Discussion

Namanereis hummelincki has been reported from other Caribbean islands; populations exist on Bonaire, Cuba, Jamaica, Hispaniola, Curaçao, Blanquilla, and Barbados (Glasby 1999, Williams 2004). Williams (2004) reported that *N. hummelincki* could be found in deep inland wells on Barbados. Given its wide distribution in the Caribbean and the suggestion that this species is very commonly collected in brackish waters in the southern Caribbean (Orensanz 1981, but see Williams 2004 for a discussion of this statement), its presence on Montserrat is not surprising.

The lack of eyes and epidermal pigmentation in this species indicates adaptation to subterranean habitats (Glasby 1999, Glasby & Timm 2008) and the specimens from Barbados (Williams 2004) were collected from a subterranean aquifer. Despite the fact that the specimens were collected in epigean waters, the effort required to locate a second specimen after the first specimen was encountered, in addition to the local rarity of the sediment in which they were found, suggests that they are rare in that habitat. The Nantes River specimens were probably waifs; washed out of the springs that feed the river from an elevated groundwater aquifer (Hemmings *et al.* 2015). Collecting in the springs or the subterranean waters that feed Montserrat's streams may well produce many more specimens.

Williams (2004) discussed the question of how these primarily subterranean freshwater species might have colonized Barbados. Two possible scenarios proposed were through ancient continental connections or through human-mediated activities, such as drilling wells. The presence of *N. hummelincki* on Montserrat raises the same questions.

Following Glasby's (1999) revision of the Namanereidinae, *N. hummelincki* forms a small clade with *Namanereis cavernicola* (Solís-Weiss & Espinasa), *Namanereis minuta* Glasby, and *Namanereis stocki* Glasby, having at least 30 morphological character states in common, including absent eyes and a jaw with a bifid terminal tooth. The jaw morphology of the Montserrat specimen, while generally similar to the description and illustration provided in Glasby (1999), differs in having a wide ventral protrusion proximal to the terminal bifurcation (Fig. 3). Glasby (1999) mentions that specimens of *N. hummelincki* occasionally have a single subterminal tooth, but he does not mention which of the specimens he examined possess such a tooth and provides no further discussion of the feature.

We hesitate to characterize this protrusion as a subterminal tooth, partially because we only examined one specimen, and thus cannot assert the protrusion as a taxonomically significant character. Because terminally bifid jaws are a synapomorphy that delineates a distinct clade within *Namanereis* by the current taxonomy of the genus (Glasby 1999), further research into the incidence of subterminal teeth in specimens identified as *N. hummeli* could have significant taxonomic and cladistic implications. If the particular jaw morphology discussed and illustrated in this paper is unique to the Montserrat population, it could indicate a long period of separation from the other island populations, and potentially a specific difference. Such a finding would also provide some support for the scenario of freshwater dispersal during continental connections to explain the Caribbean distribution of the species.

DNA data could help to resolve the unclear phylogeny of the different populations of *N. hummeli* in addition to that of the other morphologically similar polychaetes within *Namanereis*, but unfortunately there are no DNA data available from Caribbean *Namanereis* spp. to compare with the sequence from the Montserrat specimen. Such a comparison might also help determine the magnitude of the temporal separation between the different populations of *Namanereis* in the Caribbean basin.

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